VOLUME 9 (SPECIAL ISSUE 1) 2023

ISSN 2454 – 3055

Guest Editor: Dr. S. Mohanasundaram
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INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9 (Special Issue 1), 1-13 (2023)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Synthesis, Characterization and Estimation of Antibacterial Activity of Ferric Thiocyanate Nanoparticles from *Tectona grandis* Prepared by Chemical and Electrochemical Methods

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Received: 14th December, 2022; **Accepted:** 5th January, 2023; **Published online:** 22nd January, 2023

https://doi.org/10.33745/ijzi.2023.v09ispl1.001

Abstract: Chemical and electrochemical methods have synthesized ferric thiocyanate. First, Fe^{2+} was isolated from *Tectona grandis* and then oxidized by air to form Fe^{3+} ferric iron. Then it was compared to ammonium thiocyanate to make blood-red ferric thiocyanate. It was characterized by ultraviolet–visible absorption spectroscopy. The blood red solution is prepared by chemical method. The electrochemical method should only have one peak in the low wavelength region. Characterized the fluorescence absorption spectrum of the same chemical and electrochemical solutions. In the chemical method, the peaks appeared at 450 nm, 525 nm and 610 nm. Peaks appeared at 420 nm, 490 nm and 540 nm in the electrochemical method. The two samples were then characterized using FTIR spectra. The peaks of the two spectra matched each other exactly. In EDAX, two samples were observed from the bar diagram and the weight percentage of the elements present. The elements presented in the chemical method and electrochemical method were similar. SEM determined the particle size. In the chemical method, the particle size was 84.41 nm. In the electrochemical method, the particle size was 31.32nm, 47.08 nm and 39.23nm. The size of the ferric thiocyanate nanoparticle prepared by an electrochemical method was smaller than that chemical method. The electrochemical method produced spherical nanoparticles, whereas the chemical method produced wire-like nanoparticles. This research on nanoparticles is being used to find ways to improve drugs and make things less likely to rust.

Keywords: Nanoparticle, Ferric thiocyanate, UV-Visible spectroscopy, FTIR, SEM

Citation: Satish A. and Leema Rose A.: Synthesis, characterization and estimation of antibacterial activity of ferric thiocyanate nanoparticles from *Tectona grandis* prepared by chemical and electrochemical methods. Intern. J. Zool. Invest. 9(Special Issue 1): 1-13, 2023.

https://doi.org/10.33745/ijzi.2023.v09ispl1.001

Introduction

Nanotechnology might make medical and biotechnology products and processes more customized, portable, cheaper, safer, and simpler to use (Drexler, 1992; Buzea *et al*., 2007). Scientists found tiny particles, thin coatings, etc. (Adams and Baughman, 2005). They are

possessing all the distinct characteristics from bigger materials. Thus, better gadgets, architectures, and materials are limitless. Nanotechnologists create life-changing materials and technology. Nanoscale pumps, switches, and motors exist, but nanotechnology pioneers' small, self-replicating devices may never happen (Kahn and Jennifer, 2006). Semiconducting quantum dots improve biological imaging for medical diagnosis. They exhibit vivid colours when irradiated with UV light, allowing them to find and identify individual cells and biological activity. These crystals can detect light up to 1,000 times better than the standard dyes used in many biological tests, such as MRIs, and give more information. Living creatures' DNA develops structures molecule by molecule (Regan *et al*., 2005a).

Inorganic NH_4SCN is ammonium thiocyanate. Liquid ammonia, alcohol, acetone, water, ethanol, and methanol dissolve it. Ammonium thiocyanate precipitates metal ions such as copper, silver, zinc, lead, and mercury in organic solvents. NH_4SCN is a little bit acidic, and when it is mixed with caustic soda or potash, it makes sodium or potassium thiocyanate. It forms a deep-red ferric thiocyanate complex with ferric salts. Ammonium thiocyanate is used to make herbicides, thiourea, transparent artificial resins, photographic stabilizers, rust-proofing compositions, textile dyeing and printing adjuvants, oil field tracers, hafnium-zirconium separation, titrimetric analyses (Regan *et al*., 2005b).

Iron has the atomic number 26 and the symbol Fe. It is a metal from the first transition series. It's Earth's fourth most abundant element. Bivalent iron (II), or ferrous, compounds and trivalent iron (III), or ferric, compounds are chemically active. Ferric compounds contain iron. In chemistry, iron with an oxidation number of +3 is called iron (III) or Fe^{3+}, whereas iron with +2 is called iron (II) or Fe^{2+}. Rust, an insoluble iron (III) compound, is the air's most stable form of iron (Wells, 1985).

Thiocyanate anion $(SCN)^-$ is the conjugate base of thiocyanic acid. Potassium and sodium thiocyanates are colourless derivatives. Thiocyanates are SCN-containing organic compounds. Thiocyanate is a sulfur-containing cyanate ion, $(OCN)^-$. Due to its reactivity, $(SCN)^-$ is a pseudohalide. Thiocyanate is called rhodanide because of its crimson iron complexes (Earnshaw and Greenwood, 1997). Cyanide with elemental sulphur or thiosulfate form thiocyanate. When $(SCN)^-$ is introduced to a solution with iron (III) ions (Fe^{3+}), $(Fe(NCS)(H_2O)_5)^{2+}$ forms, turning the solution blood crimson. The blood-red complex pentaaqua (thiocyanato-N) iron (III), $(Fe(NCS)(H_2O)_5)^{2+}$, indicating Fe^{3+} in solution.

This study synthesized and characterized ferric thiocyanate nanoparticles. Chemical-electrochemical nanoparticle synthesis was performed. Because of their comparable physical and chemical characteristics, we synthesized ferric thiocyanate nanoparticles using ammonium thiocyanate. Fluorescence, FTIR, UV, SEM, and EDAX were used for evaluation.

Materials and Methods

Preparation and Characterization Techniques:

Silver Nanoparticle Synthesis:

After adding 10 ml of leaf extract of *Tectona grandis* to 90 ml of an aqueous solution containing 1 mM of silver nitrate, the mixture was heated at 80 °C for 3 h while being continuously stirred. The transformation from yellow to a dark brown hue served as an early indicator of the development of the AgNPs.

Preparation of ferric thiocyanate by Electrochemical method:

1g of KCNS (potassium thiocynate) was dissolved in 100 ml of water, and an undivided cell holds it. Electrolysis was done with graphite and mild steel anodes. 350 mA/cm^2 current and 6-volt potential were allowed for 5 min with stirring. Stirring caused a blood-red colour around the mild steel anode to spread throughout the fluid. The reaction stopped after 5 min. UV-visible absorption and fluorescence spectroscopy evaluated the blood-red ferric thiocyanate

solution. A few drops of fluid were dried on the glass plate. Crystal FTIR, SEM, and EDAX were recorded.

UV-VIS Spectroscopy (*UV*):

Ultraviolet–visible spectroscopy or spectrophotometry is ultraviolet-visible absorption or reflectance spectroscopy. It employs visible and nearby light. Chemical colour depends on visible light absorption or reflection. Molecules undergo electronic changes. Absorption measures ground-to-excited state transitions, whereas fluorescence spectroscopy detects excited-to-ground state transitions.

Fluorescence:

After absorbing photons, fluorescence spectroscopy assesses photon intensity. Because of its sensitivity and selectivity, fluorescence is used in many analytical sciences. It may study real-time structure and dynamics in solution and under microscopes, notably for bio-molecular systems. Fluorescence happens when a fluorophore absorbs a photon and cannot return to its ground state without releasing a photon. Emission normally begins at the ground vibrational level of the excited electronic state and ends at its excited vibrational level. Fluorescence signals arise at greater wavelengths than absorption. The fluorescence signals' energies and intensities reveal fluorophores' structures and surroundings.

FTIR:

Organic synthesis, polymer science, petrochemical engineering, pharmaceuticals, and food analysis employ FTIR spectrometers. FTIR spectrometers and chromatography can study chemical processes and unstable compounds. Molecular vibrational spectrum is infrared. Vibrational energy gap determines absorption peak frequency. The number and strength of absorption peaks depend on the molecule's vibrational freedom. Infrared absorption spectroscopy can investigate gas, liquid, and solid samples, making it more valuable. Infrared absorption spectroscopy uses 4000~400 cm^{-1} because most organic molecules and inorganic ions absorb radiation in this area.

SEM chemical analysis with EDAX is common. This approach detects X-rays from electron beam-sample interactions. Map the specimen's chemical elements. Exploratory data analysis combines compositional and morphological data. SEM-EDAX is often used to determine stone surface morphology and chemical composition.

Electron Microscopy (*SEM*):

SEM and reflecting light microscopes offer comparable data. SEMs are utilised for topography. A set of solenoids moves the beam back and forth over the sample, scanning the surface to detect secondary electrons and create a picture. SEMs create clear, black-and-white 3D pictures while being 10 times weaker than TEMs.

Ferric Thiocyanate Preparation by Chemical Method:

Iron can exist in two oxidation states namely ferrous (Fe^{2+}) and ferric (Fe^{3+}). These ions can be detected by chemical tests. When potassium ferricyanide is added to a ferrous salt (Fe^{2+}), Prussian blue is produced. When potassium ferrocyanide is added to Fe^{3+}, Prussian blue is obtained. Another test for Fe^{3+} is, it gives blood red colour with ammonium thiocyanate (NH_4CNS) solution or potassium thiocyanate solution (KCNS). The red colour is due to formation of Ferric thiocyanate (Fig. 1). The antioxidant activities of various compounds can be detected by ferric thiocyanate.

Antibacterial activity:

A 6-millimetre-thick Whatman No. 1 filter paper disc was autoclaved for 15 min at 121 °C. Samples had 1.2 x 108 CFU/ml. Impregnated discs were spaced on the medium and incubated at 37°C for 24 h. The gold standard disc contained 10 mg of streptomycin. The antibacterial activity of nanoparticles synthesized by both methods were tested against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi.*

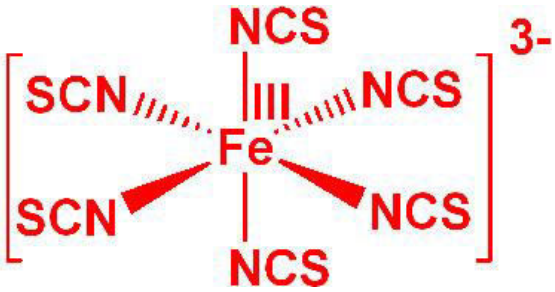

Fig. 1: Structure of blood red colored ferric thiocyanate.

Results

Silver nanoparticles synthesized from Tectona grandis and confirmed by the occurrence of dark brown colour.

Mechanism:

Chemical Method:

In chemical method, an aqueous solution of ferric chloride (Fe^{3+}) was mixed with an aqueous solution of ammonium thiocyanate. Blood red color of ferric thiocyanate was produced.

$Fe^{3+} + 6SCN^- \rightarrow (Fe(SCN)6)^{3-}$ (Blood red color)

Electrochemical Method:

When electrolysis was carried out using mild steel (iron) anode, first Fe^{2+} was produced. It was air oxidized (dissolved oxidized) to Fe^{3+}. This ferric iron compared with ammonium thiocyanate to give blood red colored ferric thiocyanate.

$$Fe \rightarrow Fe^{2+} + 2e^-$$
$$Fe^{2+} \rightarrow Fe^{3+} + e^-$$

$Fe^{3+} + 6SCN^- \rightarrow (Fe(SCN)_6)^{3-}$ (Blood red color ferric thiocyanate)

UV-*Visible Absorption Spectra*:

UV-visible absorption of red solution prepared by chemical method and electrochemical method were record in a JASCO V-630 spectrophotometer.

Ultraviolet-Visible Absorption Spectrum in Chemical Method:

The UV – visible absorption spectrum of the blood red solution prepared by chemical method of mixing aqueous solution of ferric chloride and NH_4CNS is shown in Figure 2. Peaks appear at 240 nm, 300nm, and 450 nm. The corresponding absorption spectrum (λ_{ex}=245) is shown in Figure 2. The UV-visible absorption spectrum of the blood red solution prepared by electrochemical method is shown in Figure 3.

The emission spectrum (λex=240nm) is shown in Figure 4. Peaks appeared at 300 nm, 370 nm, 420 nm, 475 nm and 530 nm. The product produced by electrochemical method showed only one peak in the low wavelength region, in the UV absorption spectrum. This may be due to the formation of nano particles of smaller size when compound with the sample prepared by chemical method. Corresponding emission spectrum is also a different one. When excited, it emitted light of different wavelengths.

Fluorescence Spectra:

Fluorescence spectra of the above solution were recorded by Agilent Technologies Cary Edipse Fluorescence Spectrophotometer. The Fluorescence absorption spectrum of the red solution prepared by chemical method is shown in Figure 4. Fluorescence spectrum of the blood red solution was prepared by mixing ferric thiocyanate solution and NH_4CNS solution. (λ_{ex}=245nm). Peaks appeared at 450 nm, 525 nm, and 610 nm. The Fluorescence absorption spectrum of the red solution prepared by electrochemical method is shown in Figure 5. Fluorescence spectrum of blood red solution was prepared by electrochemical method

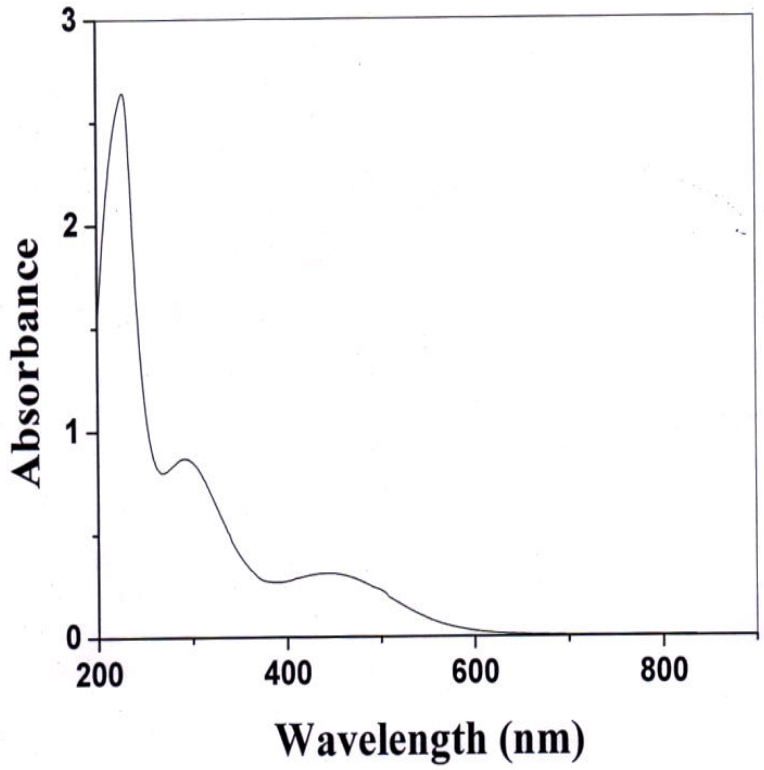

Fig. 2: Uv–visible absorption spectrum of Blood red solution (ferric thiocyanate) prepared by chemical method.

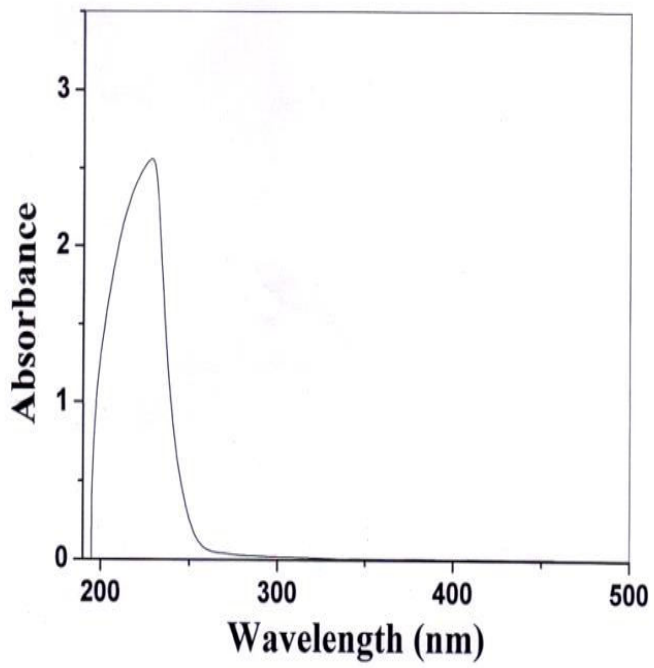

Fig. 3: UV–visible absorption spectrum of blood red solution prepared by electrochemical method.

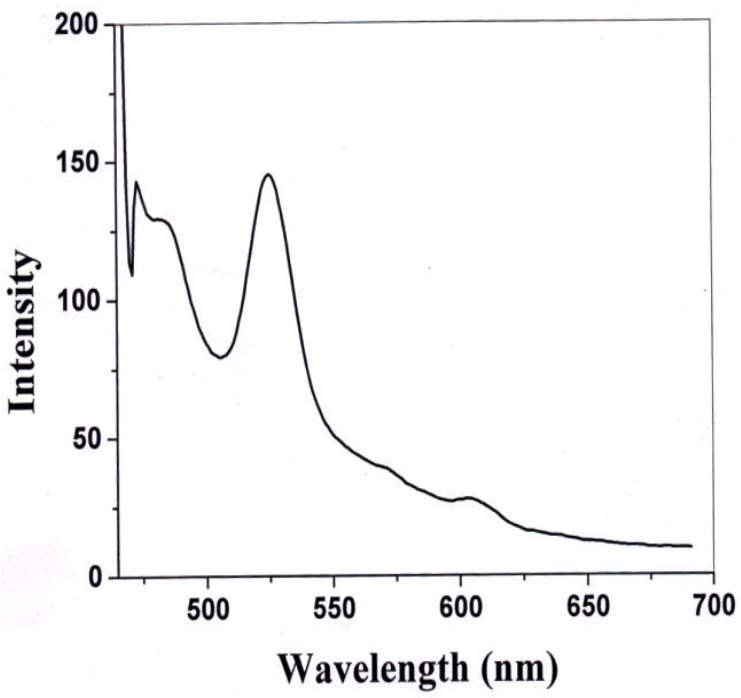

Fig. 4: Fluorescence spectrum of Blood red solution prepared by chemical method.

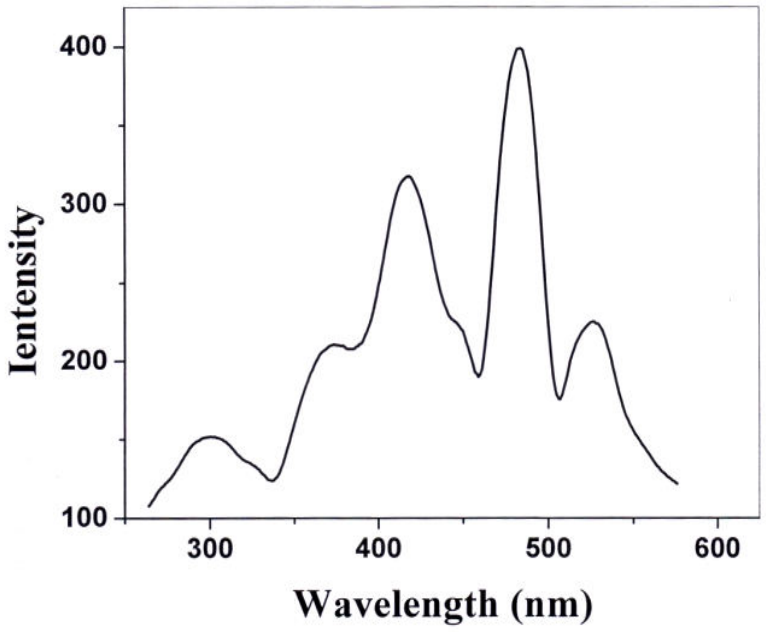

Fig. 5: Fluorescence spectrum of Blood red solution prepared by electrochemical method.

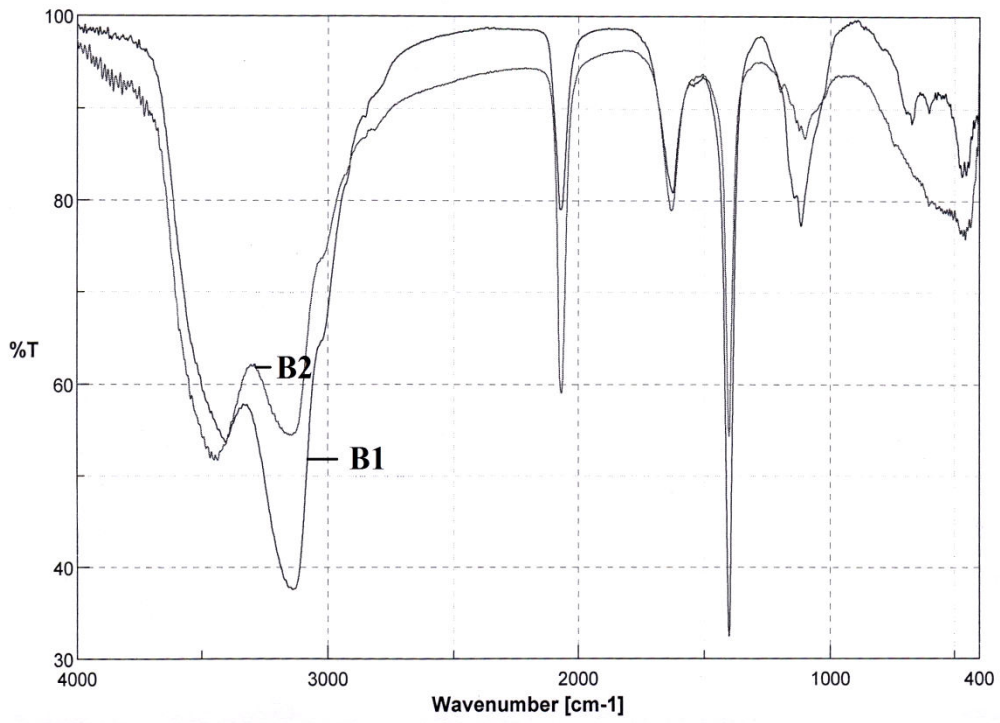

Fig. 6: FTIR spectra of ferric thiocyanate. B1 prepared by chemical method and B2 prepared by electrochemical method.

(λ_{ex}=240nm). Peaks appeared at 420 nm, 490 nm and 530 nm.

FTIR Spectra and EDAX:

A few drops of each red solution were dried on glass plates. Dried crystals were used for recording FTIR spectra and EDAX.

FTIR Spectra:

A few drops of the red solution prepared by chemical method and electrochemical method were placed on glass plates and dried. Red crystals were obtained. FTIR spectra of the two samples were recorded (KBr). They are shown in Figure 6. It is interesting to note that the peaks of the two spectra matched each other exactly. This suggests that the two compounds prepared by two different methods are very similar or identical. But they give different UV spectra and Fluorescence spectra. This may be due to the difference of the particles size. B_1 prepared by chemical method and B_2 prepared by electrochemical method.

Analysis EDAX by Chemical Method:

Ferric thiocyanate (Blood red color) was prepared by mixing of ferric chloride solution and ammonium thiocyanate solution. A few drop of blood red color formed was placed on a clean glass plate and dried overnight. The SEM image (Fig. 7) and EDAX is shown in Figure 9. The weight percentage and atomic percentage of the elements present in the film are given in Table 1.

EDAX of Ferric Thiocyanate Prepared by Electrochemical Method:

The SEM image of ferric thiocyanate prepared by electrochemical method is shown in Figure 8. The EDAX of ferric thiocyanate prepared by electrochemical method is shown in Figure 10. The weight percentage and Atomic percentage of various elements present in the ferric thiocyanate, prepared by electrochemical method are given in Table 2.

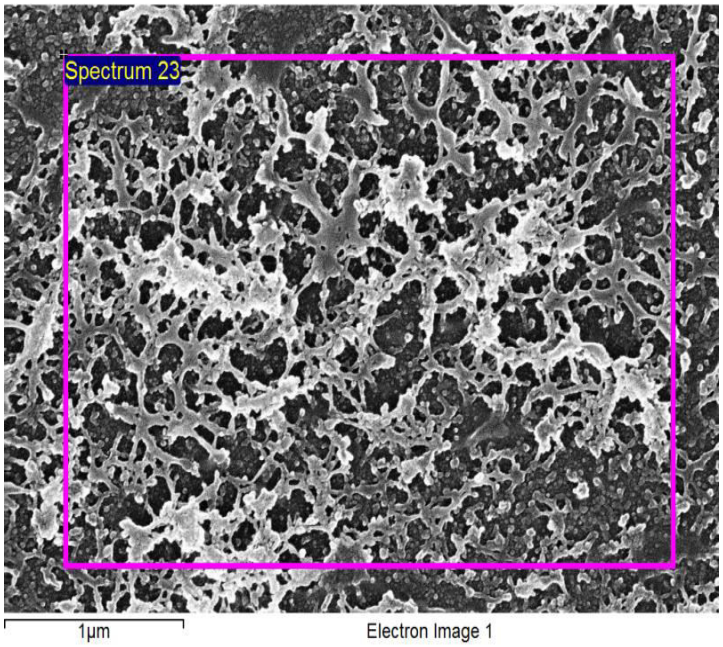

Fig. 7: SEM image of ferric thiocyanate prepared by electrochemical method.

Table 1: Weight percentage and Atomic percentage of elements prepared by chemical method.

Elements Present	Weight %	Atomic %
C K	10.90	16.31
N K	16.93	21.72
O K	34.22	38.42
Na K	5.66	4.42
Mg K	1.76	1.30
Si K	19.95	12.76
S K	4.97	2.78
Cl K	0.50	0.25
K K	0.26	0.12
Ca K	2.84	1.27
Fe K	2.00	0.64
Totals	100.00	

Table 2: Weight percentage and Atomic percentage of elements prepared by electrochemical method

Elements Presents	Weight (%)	Atomic (%)
C K	5.24	8.47
O K	48.94	59.32
Na K	8.33	7.03
Mg K	1.90	1.51
Al K	0.53	0.38
Si K	30.60	21.13
Ca K	4.46	2.16
Totals	**100.00**	**100.00**

Scanning Electron Microscope Image (*Prepared by Chemical Method*):

The SEM image of ferric thiocyanate prepared by chemical method in shown Figure 11. Minute nano wires of ferric thiocyanate is shown in Figure 12. Nano particles size is about 84.41nm; Length of the nano wires are 12.66 nm, and 71.87 nm.

It is seen from Figure 11 that nano wires are deposited on rock like surfaces. In this figure it is observed that these nanowires are crowed unlike in Figure 12 where nanowires throughout the area.

Scanning Electron Microscope Image (*Prepared by Electrochemical Method*):

The minute nanospheres of the compound are shown in Figure13. Nanoparticle sizes are about 31.32 nm, 47.08 nm and 39.23 nm. It has also been seen that nanoparticles stick to each other because they are similar. Interestingly, the size of nanoparticles prepared by the electrochemical method is smaller than those prepared by the chemical method. The layer with the interlocking networks can be seen on the top of the film (Fig. 14). Below this network, spherical nanoparticles of Ferric thiocyanate are seen.

Antibacterial Activity:

The minute nanospheres of the compound are shown in Figure 13. Nanoparticle sizes are about 31.32 nm, 47.08 nm and 39.23 nm. It has also been seen that nanoparticles stick to each other because they are similar. Interestingly, the size of nanoparticles prepared by the electrochemical method (Fig. 13) is smaller than those prepared by the chemical method. The layer with the interlocking networks can be seen on the top of the film (Fig. 14). Below this network, spherical nanoparticles of Ferric thiocyanate are seen. Nanoparticles synthesized by both the chemical and electrochemical methods have inhibiting capacity against all the three microbes *E. coli*, *S.aureus* and *S.typhi* (Table 3).

Discussion

Main purpose of the present work was to synthesize ferric thiocyanate by electrochemical method and to characterize it by UV-visible absorption spectroscopy, fluorescence spectroscopy, and FTIR spectroscopy. The surface morphology and size of the particles can be

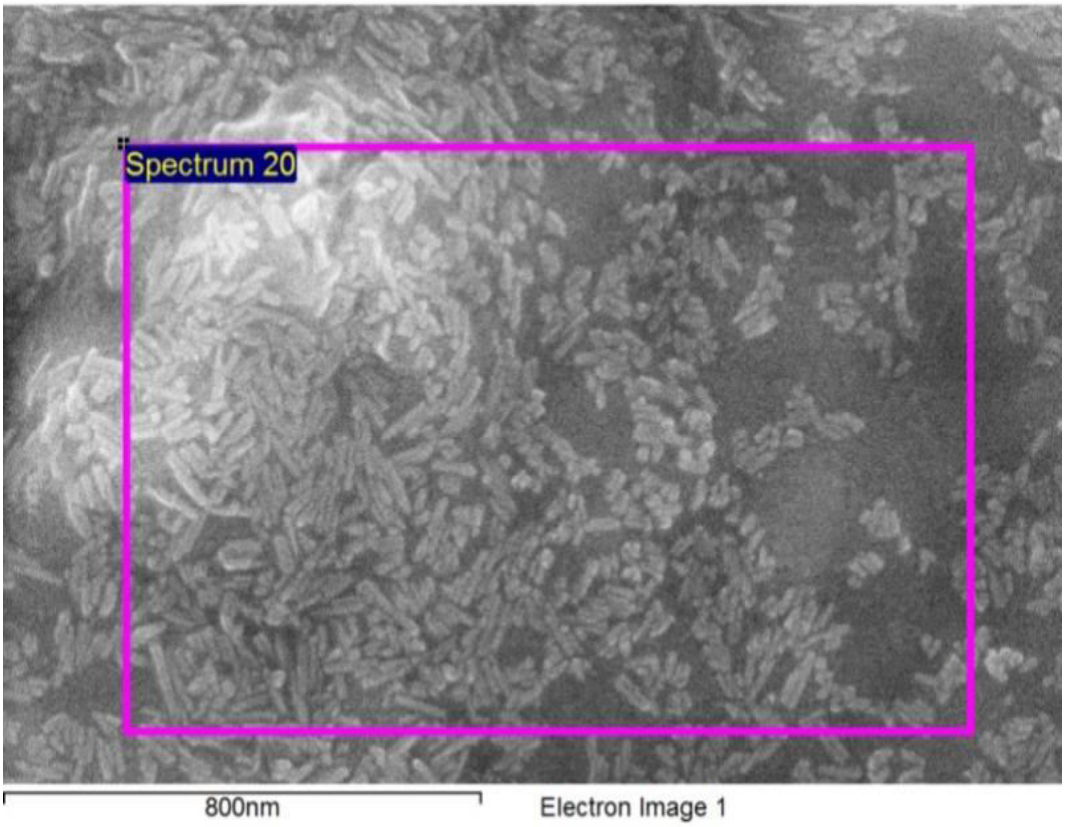

Fig. 8: SEM image of ferric thiocyanate prepared by chemical method.

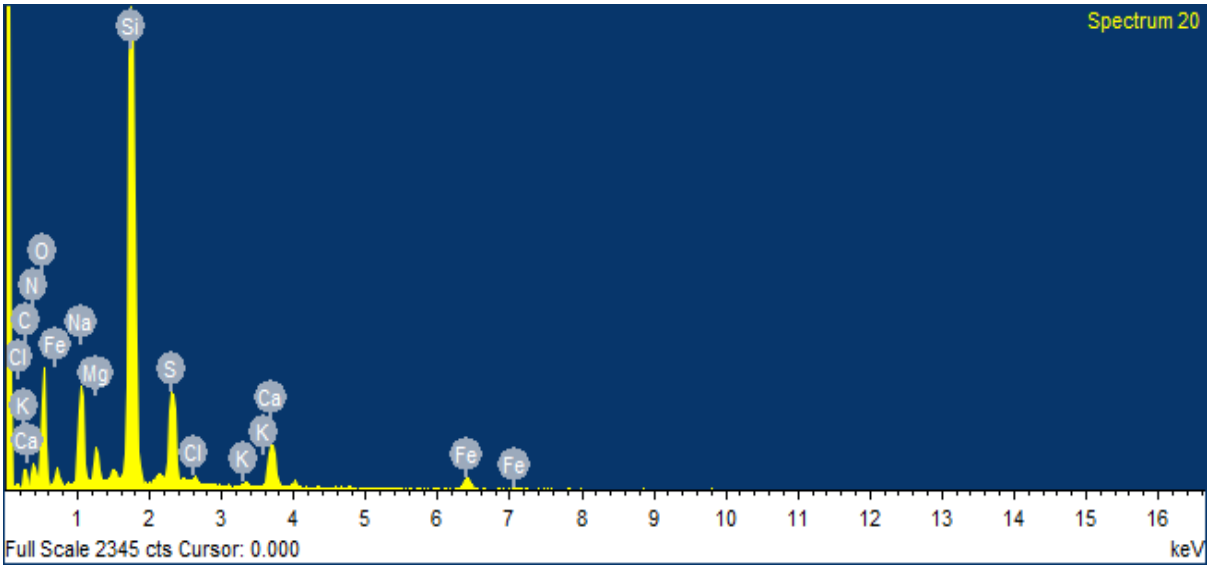

Fig. 9: EDAX spectrum of ferric thiocyanate prepared by chemical method.

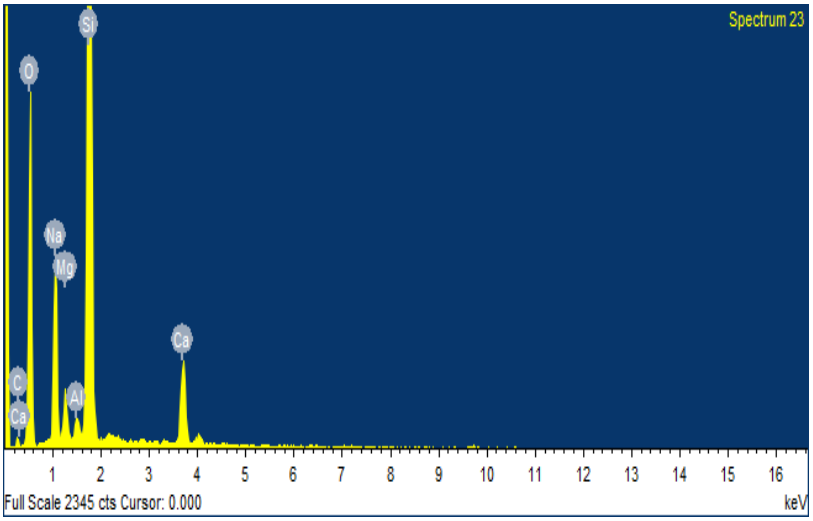

Fig. 10: EDAX spectrum of ferric thiocyanate prepared by electrochemical method.

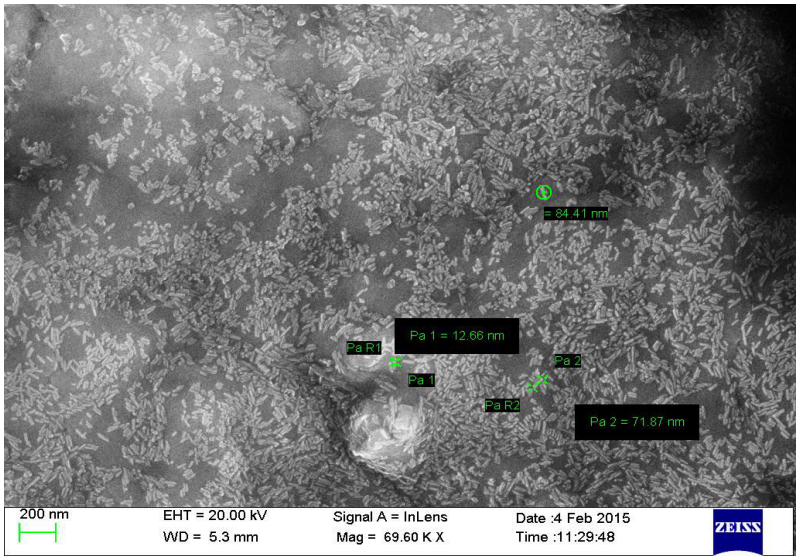

Fig. 11: SEM image of ferric thiocyanate prepared by chemical method.

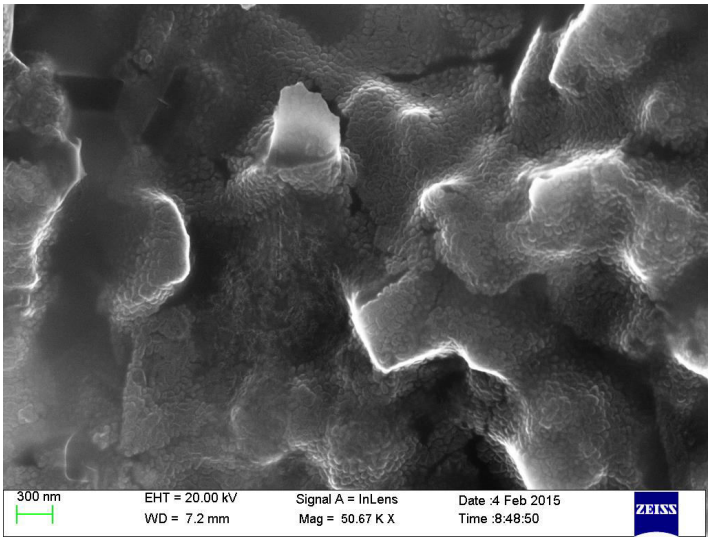

Fig. 12: SEM image of nano wires of ferric thiocyanate.

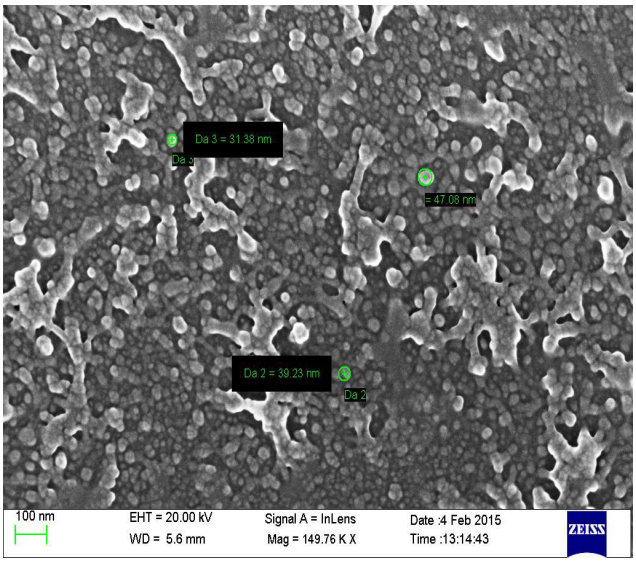

Fig. 13: SEM image of ferric thiocyanate prepared by electrochemical method.

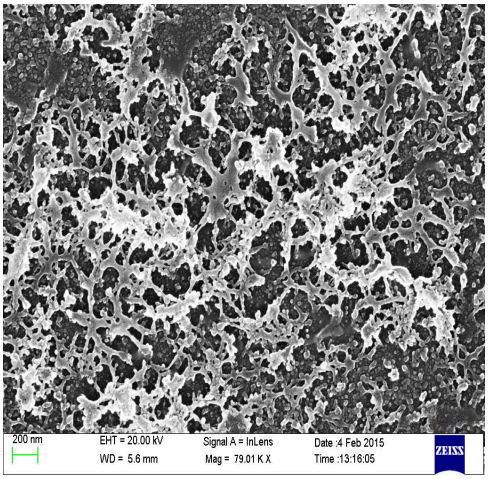

Fig. 14: SEM image of upper surface of ferric thiocyanate.

Table 3: Antibacterial Activity Against Pathogenic Bacteria

Organism	Escherichia coli	Staphylococcus aureus	Salmonella typhi
NP – Chemical	+ +	+ +	+ +
NP-Electrochemical	+ +	+ +	+ +

investigated by SEM and EDAX. When Fe^{3+} react with SCN^- ion, blood red colour is produced. During electrolysis in an aqueous medium when mild steel is used as an anode iron goes into solution and Fe^{2+} will be produced. Automatically it will be oxidized to Fe^{3+}. If NH_4CNS solution is taken in the environment, and Fe^{3+} and SCN^- will react then blood red colour (ferric thiocyanate) will be produced. The cathode can be graphite, because it will not interfere with the main reaction. The ferric thiocyanate produced can be compared with that produced by mixing ferric

chloride and NH4CNS aqueous solution.

At ambient temperature, Fe_2O_3 and Fe_3O_4 NPs have distinct ferrimagnetism. Long-term particle aggregation and air oxidation reduce IONP dispersity and magnetism. For nanomedicine applications, NPs are stabilised in an inert environment and made water-soluble at physiological pH (Wu *et al*., 2015). Magnetic iron oxide NPs have high surface energies due to their huge surface-to-volume ratio. They agglomerate to reduce surface energy. Naked iron oxide NPs have significant chemical activity and readily oxidize in air (particularly magnetite), losing magnetic and dispersibility. Thus, magnetic iron oxide NPs must be protected by adequate surface coating and effective techniques.

Protein, polypeptide, antibody, biotin, and avidin are only some of the biological molecules that may be connected to iron oxide NPs either directly or indirectly through chemical connections at functional end groups to increase their specificity. The biocompatibility of iron oxide NPs will be greatly improved by their functionalization with biomolecules. Proteins, DNA, cells, biological products, and so on may all be more easily isolated with the aid of magnetic NPs (Lewin *et al*., 2000).

Conclusion

The ferric thiocyanate nano particle size that is created by the electrochemical technique is smaller than the size that is prepared by the chemical approach. The form of the nanoparticles that were created by the electrochemical technique was spherical. The wire-like structure of the nano particle was due to the chemical way of production. The size of nanoparticles may fluctuate from around 31.32 nm to 47.08 nm and then back down to 39.23 nm. It has also been shown that nanoparticles of a similar size may adhere to one another. Nanoparticles synthesized by both the chemical and electrochemical methods have inhibiting capacity against all the three microbes *E. coli*, *S. aureus* and *S. typhi*.

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